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Transmission and distribution of cassava brown streak virus disease in cassava growing areas of Kenya

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ABSTRACT

Objectives: To determine the incidence and distribution of cassava brown streak disease (CBSD) in relation to *Bemisia tabaci* populations in Central, Eastern, Nyanza and Western provinces of Kenya and investigate the ability of 4 cassava pests to transmit cassava brown streak virus (CBSV).

Methodology and results: A multistage sampling survey was conducted from November 2006 to April 2007 to determine CBSD incidence and distribution in relation to *B. tabaci* populations in major non-coastal cassava growing areas of Kenya. In a separate study, adults of test insects were allowed 48 hours acquisition feeding period on CBSV infected cassava plants in cages before being transferred to uninfected plants. CBSD was present at high incidences in western Kenya (38-93%) but was not detected in Central and Eastern provinces. Large *B. tabaci* populations observed in western Kenya were significantly and positively correlated to CBSD incidence indicating a considerable contribution of the whiteflies to the spread of CBSD. Transmission of CBSV by *B. tabaci* occurred at 27.8%, a confirmation and additional evidence that this whitefly species is a vector of CBSV.

Conclusion and application: This report provides evidence that CBSD is no longer restricted in distribution to the coastal lowlands of Kenya. The study further confirms that CBSD is spread by *B. tabaci* and therefore its control should be included in the overall management of CBSD. These findings increase the understanding of CBSD epidemiology.

Key words: CBSD, incidence, transmission, *Bemisia tabaci*

INTRODUCTION

Cassava, *(Manihot esculenta Crantz)* is an important crop in Kenya, grown for both food and income on approximately 77,502 ha with an annual output of 841,196 tons (FAO, 2007). Approximately 60% of this production is in Western Kenya, 10% in Eastern province and 30% in Coast province (Crop Crisis Control Project, 2006). Cassava production is constrained by many biotic factors of which cassava mosaic geminiviruses (CMGs) and cassava brown streak virus (CBSV) are the major threats. The cassava brown streak disease (CBSD) is caused by CBSV, a member of the

Potyviridae family and genus Ipomovirus (Monger *et al.,* 2001). The disease causes economic losses resulting from damage to the above ground parts characterized by leaf chlorosis and stem lesions with complete die back as well as the spoilage of roots due to dry corky necrotic rot on starchy tissues (Hillocks, 1999; Hillocks *et al*., 2003). CBSD has been reported to cause up to 70% yield loss by reducing the root sizes and causing pitting and constriction on roots (Hillocks *et al*., 2001). Necrotic lesions and/or discoloration of the roots due to infection render them unpalatable and unmarketable, and this explains most of the quantitative and qualitative losses (Nichols, 1950).

Since its first description in northern Tanzania (Storey, 1936), CBSD has been endemic in the East African coastal areas from southern Kenya, through to the Zambezi river in Mozambique. The disease also occurs in some inland areas of Malawi and Uganda, up to altitudes of 1000 m (Nichols, 1950; Sauti & Chipungu, 1993; Legg & Raya, 1998; Hillocks *et al*., 2002). Although CBSD distribution has been confirmed in Tanzania, Mozambique, Malawi and coastal Kenya (Hillocks *et al*., 2002) its occurrence in other cassava growing regions of Kenya has not been established. Since it is known that CBSD symptoms can be expressed at altitudes greater than 1000m when infected cuttings are planted it is possible that the disease has spread beyond the coastal lowlands of Kenya (Alicai *et al*., 2007). CBSD re-emergence and widespread occurrence has been reported at high incidence (64%) in areas where it was endemic or absent in Uganda (Alicai *et al.,* 2007). The emergence of CBSD in higher altitude areas as observed in Uganda has led to a shift in the general belief that the disease is delimited in distribution mainly along the coastal lowlands. Whereas CBSD is widely spread in coastal Kenya (Munga *et al*., 2002), information on its occurrence, spread and distribution in inland cassava growing regions of Kenya is lacking.

CBSV is transmitted by graft between cassava plants (Storey, 1936) and mechanically from cassava to a number of herbaceous hosts (Lister, 1959). It is also insect-transmitted by the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) (Storey, 1939; Bock, 1994), but this had not been supported with direct evidence until recently when low transmission rates (20-22%) by *B. tabaci*, were achieved (Maruthi *et al.,* 2004). The transmission rates are inconsistent with high CBSD incidences (64%) observed in field surveys (Alicai *et al.,* 2007), Since cassava is grown in altitudes higher than 1000m in Kenya where the vector is widely distributed, the disease may have spread to areas where it has not been reported before. Different arthropod pests infest cassava but whether or not they contribute to CBSD spread is unknown.

This study determined the distribution of CBSD in non-coastal cassava growing areas and investigated the potential of four key cassava pests to transmit CBSV. The pests are *B. tabaci* (Gennadius), *Mononychellus tanajoa* (Bondar), *Phanococus manihoti* and *Tetranychus urticae*.

MATERIALS AND METHODS

CBSD survey: A survey was conducted between November 2006 and April 2007 to determine CBSD incidence, prevalence and adult whitefly abundance in major cassava growing areas of Central, Eastern, Nyanza and Western provinces of Kenya.

Sampling was done when the crops were about 6 to 9 months old when CBSD symptoms are clearly visualised and the whitefly *B. tabaci* has a preferential feeding. The sampling was conducted following existing district administrative boundaries further sub-divided into divisions (Otim-Nape *et al.,* 2000). The procedure involved stopping at regular predetermined intervals of about 2-5km (to allow for wide coverage of the survey area) between farmer fields along the major motorable roads traversing each sampling location.

CBSD incidence was estimated by determining the number of plants within a field that showed CBSD symptoms expressed as percentage of the total number of plants observed. On the other hand, prevalence of CBSD was determined as the proportion in percentage of production units (farmer fields) in which the disease symptoms were observed.

A total of 30 plants were randomly sampled along the diagonals of each field to determine CBSD incidence. Adult whitefly populations were determined

by counting the number of whiteflies on the five top most expanded leaves of a representative shoot on the 30 cassava plants randomly selected along diagonals of each field (Sseruwagi *et al.,* 2004)

Severity on shoot symptoms was recorded following a scale of 1 to 5 (Hillocks *et al*., 1996) where: 1- no apparent symptoms; 2- slight foliar mosaic, no stem lesions: 3- foliar mosaic, mild stem lesions no die back; 4- foliar mosaic and pronounced stem lesions no die back; 5- defoliation with stem lesions and pronounced die back.

Asymptomatic and symptomatic leaf samples were collected and tested for presence of CBSV using reverse transcriptase polymerase chain reaction (RT-PCR) (Monger *et al.,* 2001). Data on CBSD incidence and *B. tabaci* populations were subjected to analysis of variance and the means separated by the Least Significant Difference test at P=0.05 before performing correlation analysis.

CBSV transmission: To determine the ability of cassava pests to transmit CBSV, transmission trials were set up using adult whiteflies (*B. tabaci*), cassava green mites (*M. tanajoa*), red spider mites (*T. urticae*) and cassava mealy bugs (*Phanococcus manihoti*). Two susceptible cassava varieties (MM96/5280 and MM96/4466) confirmed to be CBSV-free by reverse transcriptase polymerase chain reaction (RT-PCR) were used (Monger *et al.,* 2001. The cultivars were selected during the survey based on CBSD incidence and whitefly population recorded on them alongside farmer preference. The two cultivars were the most popular and had highest CBSD incidence and *B. tabaci* population in western Kenya. Approximately 600 (per cage) adults of *B. tabaci* collected from cassava fields were allowed 48 hours acquisition feeding period on CBSV infected plants in cages before being transferred to uninfected plants. A hundred adults of each of the other insect species namely cassava green mite, cassava mealy bugs and red spider mites were allowed acquisition access feeding on CBSV infected plants and then transferred onto CBSV free recipient cassava plants in separate cages. Four cages each containing 6 young CBSV-free plants were established per insect species. Symptom development was monitored daily through visual observations followed by testing by RT-PCR 60 days later (Monger *et al.,* 2001). The rate of transmission was calculated as the number of infected recipient plants expressed as a percentage of the total number of recipient plants used. The experiment was repeated three times for each insect species.

RESULTS AND DISCUSSION

Symptomatology: In the farmers' fields symptoms typical of CBSD were observed on the leaves and stems which comprised foliar mosaic with a characteristic pattern of feathering along the veins and chlorotic blotches. There was a vast range of cassava cultivars grown by farmers (Table 1).

The foliar symptoms were characteristic of CBSD and indicated the presence of the disease in Western and Nyanza provinces. There was incidence variability among varieties depicting differential cultivar sensitivity to CBSD. Cultivars MM96/5280 (100%), MM96/4466 (100%) and Ex Uganda (100%), appeared most susceptible with distinct CBSD symptoms whereas cv. Migyera and some local cultivars showed mild CBSD symptoms. Cultivars Nyakatanegi and Ngungume had no symptoms. These observations concur with earlier work by Hillocks *et al.* (2001).

CBSD prevalence, incidence and severity: The data collected indicates high CBSD incidences in Bondo and Busia districts and moderate incidences (Table 2) in Teso and Siaya, with significant ($F_{[8, 74]}$ =16.5, p<0.001) differences between the districts. The CBSD incidences recorded in Western Kenya were typically high (64%) and comparable to those reported in central and Eastern Uganda which borders Western Kenya (Busia) (Alicai *et al.,* 2007).

CBSD symptom severity varied significantly (P>0.001) between the districts and was highest in Bondo (3) followed by Bungoma and Busia with a severity level of 2 (Table 2). The variation in disease incidence and severity could perhaps be due to the distribution of *B. tabaci,* the already reported CBSV vector, in addition to the differences between cultivars grown in the different districts alongside a high inflow of cassava cuttings from Uganda where CBSD outbreak has been reported (Alicai *et al.,* 2007).

Apparently the most preferred and dominant cultivars were the most affected with high CBSD incidences. According to the farmers, those cultivars that are bitter in taste (high level of cyanide) were not seriously affected by the CBSD. Upon closer observation, such cultivars also had very low whitefly populations. For instance farmers reported that they preferred M96/5280 due to its sweet taste, early maturity (9-12 months) and being ideal for fresh consumption. The reason the less susceptible cultivars are not preferred is bitterness and the long maturity period (two years for Ngungume and Nyakatanegi). It is

possible that *B. tabaci* has low preference for these cultivars due to their bitterness.

The widespread nature of CBSD may be due to the fact that the most preferred and dominant cultivars are susceptible, which presents a great challenge in disease management. More efforts needs to be directed towards breeding cassava for resistance by incorporation desirable traits from the resistant cultivars.

Table 1: Cassava Brown Streak Disease incidence and severity on predominant cassava cultivars in different cassava growing districts of Kenya.

District	Predominant cultivar	No. of farms	No. of plants	Cassava brown streak disease	
				Severity	Incidence (%)
Bondo	MM96/5280	14	420	2-4	100
Siaya	MM96/5280	4	120	2-3	100
Busia	MM96/5280	7	210	2-3	100
Teso	MM96/5280	6	180	2-2	100
Siaya	Migyera	10	145	1	0
Busia	Migyera	6	192	1-2	12.8
Teso	Migyera	10	300	1-2	21
Bungoma	Migyera	1	17	1-2	0.6
Embu	Muerisheri	11	330	1	0
Busia	MM96/4466	1	13	2-3	100
Busia	Nase4	1	55	2	3.3
Embu	Ndolo	10	30	1	0
Embu	Nguche	10	300	1	0
Busia	Magana	5	300	1-2	18
Busia	Ngungume	5	210	1	0
Bondo	Nyakatanegi	3	180	1	0
Siaya	Adhiambo lera	7	57	1	0
Busia	Ex-Uganda	1	30	2-4	100

A total of 30 plants were sampled per field. However, there were fields that had mixed varieties with some varieties having less than 30 plants along the diagonal.

Table 2: Incidence (%), prevalence (%), severity of Cassava Brown Streak Disease and mean whitefly (*Bemisia tabaci*) counts in selected cassava growing districts in Kenya.

Survey region		Cassava brown streak disease			*B. tabaci*
Province	District	Prevalence (%)	Incidence (%)	Severity**	***Mean counts
Western	Busia	87.5	60^{b} (52.45)*	2 (1-3)	1.7 ± 0.396
	Bungoma	20	6^{c} (6.58)	2 (1-2)	2.3 ± 0.396
	Teso	88	48^{b} (43.46)	1 (1-3)	2.92 ± 0.499
Nyanza	Bondo	100	93^{a} (84.49)	3(1-4)	6.77 ± 0.885
	Siaya	50	38^{b} (33.24)	1(1-3)	2.2 ± 0.712
	Kisii	0	0^{c}	1	0
Eastern	Embu	0	0^{c}	1	0.7 ± 0.367
	Machakos	0	0^{c}	1	0.5 ± 0.432
	Mbere	0	0^{c}	1	0
Central	Kirinyaga	0	0^{c}	1	0.5 ± 0.432
	Thika	0	0^{c}	1	0.7 ± 0.367

Means followed by the same letter in the incidence column are not significantly different at 5% significant level; *% incidence was arc sine transformed; **Severity of foliar CBSD symptoms was determined following a scale of 1 to 5 (Hillocks *et al.,* 1996). Figures in parenthesis are severity range; ***Figures are means of whitefly adults per 5 top most leaves.

Population of whitefly on cassava: The number of adult whiteflies per plant differed significantly (P>0.05) among the various districts surveyed and was highest (6.8) in Bondo where CBSD incidence was also high, and was lowest (0.5) in Kirinyaga where CBSD was absent (Table 2). Very low whitefly populations were recorded with majority of fields having no whiteflies although the plants were over one year old in Kisii, Embu, Machakos, Mbeere, Kirinyaga and Thika districts. However, high whitefly populations in younger plants aged 3 to 4 months with an average population of 5 adult whiteflies on each of the 5 top most leaves was recorded in some fields in Western Kenya depicting a preferential feeding habit of the whiteflies on younger apical leaves. *B. tabaci* adults have a preferential feeding and oviposition on the topmost immature leaves (Fargette, 1985). There was a significant and positive correlation (r=+0.6977, p<0.001) between number of adult whiteflies, *B. tabaci* and CBSD incidence depicting a possible role of whiteflies in the spread of CBSD. The CBSD incidence was observed to be higher where the whitefly adult numbers were high. Significant increase in the whitefly numbers seemed to lead to a greater CBSD incidence.

RT-PCR analysis: Cassava leaves with suspected CBSV infection obtained from Western, Eastern and Central provinces were collected for testing. The RT-PCR results confirmed the presence of CBSD in cassava leaves collected from western Kenya and absence in those from Eastern and Central Kenya. These data indicated absence of the virus within eastern and Central Kenya regions as at the time of this survey.

CBSV transmission by cassava pests: The positive correlation between the CBSD incidence and the number of adult whiteflies (Table 2) suggested a possible contribution of the whiteflies to the spread of CBSV. Similar findings have been reported in previous work in which super abundance of *B. tabaci* seemed to enhance the spread of CBSD in Uganda (Alicai *et al.,* 2007; Pheneas & Legg, 2007). Moreover in the transmission trials, 3 of the 12 (25%) cassava plants of cultivar MM96/5280 inoculated with CBSV using 600 adult *B. tabaci* produced typical CBSD symptoms. In the repeat experiment, 4 out of 12 (33.3%) and 3 of 12 (25%) recipient plants of cultivars MM96/5280 and MM96/4466, respectively, became infected (Fig 1, lane 3, 4 and 7). This demonstrates a considerable role of *B. tabaci* (overall rate of 27.8%) in the spread of CBSD and concurs with the results reported by Maruthi *et al.* (2004). No CBSV transmission was achieved when cassava green mites (*Mononychellus tanajoa*), red spider mites (*Tetranychus urticae*) and cassava mealy bug (*Phanococcus manihoti)* were used indicating their inability to transmit CBSV (Fig 1 lanes 6, 8).

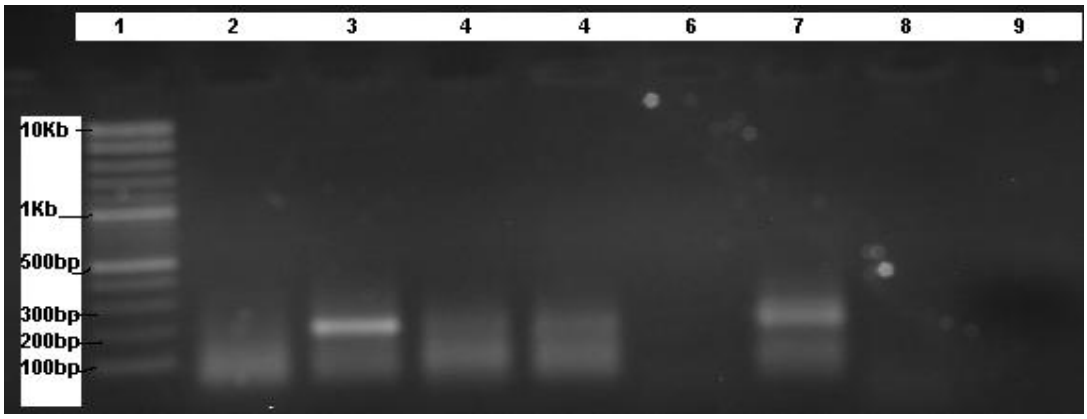

Figure 1: Photograph of PCR products showing CBSV diagnostic bands (231 base pairs) lane 1 is a 10kb DNA ladder whereas lanes 3, 4 and 7 indicate transmission by *B. tabaci* , lanes 6 and 8 indicating no transmission by cassava green mites and cassava mealy bugs; lane 9 is CBSV-free cassava leaf used as a negative control. Lane 4 was inadvertently repeated.

The fact that cultivars bred for tolerance against cassava mosaic geminiviruses such as MM96/5280 and MM96/4466 (promoted for management of CMD pandemic in western Kenya (Obiero *et al.,* 2007; Pheneas and Legg, 2007) are the ones affected by CBSD compounds the losses already being caused by CMD because CBSD causes the edible cassava roots to become corky and inedible (Hillocks, 1999 & Hillocks *et al* 2003). Similar challenges have been reported in Uganda where CBSD infection was highest in varieties that were being promoted due to their resistance to CMD (Pheneas & Legg, 2007).

The moderate to high prevalence of CBSD in all the districts surveyed in Western Kenya indicates increasing distribution of the disease in the region. It is conceivable that the disease could be present in districts that were not surveyed but border the ones covered in this region. Hence a more extensive diagnostic survey for CBSD should be carried out in the western Kenya region.

The transmission rates achieved under controlled conditions in this study are low compared to the high CBSD incidences observed in the fields, and as reported by Alicai *et al* (2007). It is likely that there are other cassava pests that transmit CBSV, apart from those tested in this study. Future studies should focus on the testing of other cassava infesting whitefly species for their ability to transmit CBSV. Another key factor in disease spread could be the continuous use of diseased planting material.

In this paper we report the first detailed survey on the extent of CBSD spread beyond the coastal lowlands of Kenya. The emergence and distribution of CBSD in 5 districts of western Kenya, a region outside the coastal belt is shown supporting the work by Pheneas and Legg (2007) which reported an outbreak of CBSD in a multiplication plot in Yala swamp of Western Kenya and confirm *B. tabaci* as a vector of CBSV. CBSD is no longer restricted in distribution along the coastal low lands of Kenya as previously known. The findings achieved so far reveal the need for CBSD management strategies to include measures that target both the disease and the whitefly. In this regard, host-plant resistance to both CBSD and the whitefly species is envisaged as a dual control strategy.

In conclusion, cassava breeding for resistance should also focus on both cassava mosaic and CBSV diseases. The cultivar sensitivity to CBSD reported here needs further trials to ascertain the reaction of farmer preferred cassava cultivars to CBSV particularly in Nyanza and Western provinces where the disease is present. This would help in identifying locally adapted cultivars with low sensitivity to the disease which can then be cleaned of virus and distributed to high risk areas such as Western and Nyanza regions of Kenya.

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